

1982

## Association of DNA With Nuclear Estradiol Receptors Released From Chromatin

Thresia Thomas  
*University of Minnesota*

Benjamin S. Leung  
*University of Minnesota*

Follow this and additional works at: <https://digitalcommons.morris.umn.edu/jmas>



Part of the [Biology Commons](#)

---

### Recommended Citation

Thomas, T., & Leung, B. S. (1982). Association of DNA With Nuclear Estradiol Receptors Released From Chromatin. *Journal of the Minnesota Academy of Science*, Vol. 48 No. 1, 6-9.  
Retrieved from <https://digitalcommons.morris.umn.edu/jmas/vol48/iss1/4>

This Article is brought to you for free and open access by the Journals at University of Minnesota Morris Digital Well. It has been accepted for inclusion in Journal of the Minnesota Academy of Science by an authorized editor of University of Minnesota Morris Digital Well. For more information, please contact [skulann@morris.umn.edu](mailto:skulann@morris.umn.edu).

# Association of DNA With Nuclear Estradiol Receptors Released From Chromatin

THRESIA THOMAS,\* BENJAMIN S. LEUNG\*\*

**ABSTRACT**—A cell-free system was established to study the role of steroid hormones in transcriptional control. The system consists of hormone-receptor-chromatin complex formation using partially purified receptor and chromatin. Micrococcal nuclease digestion of the complex released a 7S form of receptor. The absorbance at 260 nm also showed a peak in the 7S region of the sucrose gradient. DNAase I digestion caused the 7S receptor to shift to 2.8S form, while RNAase had no effect. When the receptor-chromatin complex was digested with DNAase I, different forms of receptors were observed, depending on the digestion time. Digestion of one minute produced a heterogeneous population of receptors ranging from 3S to 7S. Prolonged digestion caused a stepwise reduction in receptor size. These results suggest that 7S receptor contains an associated DNA fragment, and the transcriptional control mechanism involves binding of the steroid receptor with DNA of active chromatin.

Each cell in a multicellular organism contains identical genetic information. Yet, as a result of differentiation, a type of cell produces a specific set of proteins. For example, blood cells are programmed to produce haemoglobin while cells in pancreas produce insulin. One of the mechanisms to achieve this differentiation is transcriptional control. Only certain genes in a cell are transcribed to messenger RNA and then translated to proteins. In mammals, for example, only about 10 percent of the total DNA of the cell is ever translated to proteins. The rest of the DNA consists of repressed genes or noncoding sequences.

There are some conditions under which a repressed gene is activated. For example, neoplastic cells produce various proteins that are suppressed in normal cells. Gene activation also occurs in normal cells under the control of different regulatory proteins.

The spurt of growth at puberty occurring under the influence of sex hormones is largely due to activation of suppressed genes (O'Malley *et al*, 1977, Mathis *et al*, 1980).

The actions of estradiol and other steroid hormones are mediated by hormone receptors which are present in target tissues. The receptors are proteins which bind to these hormones with high affinity. This specific binding causes the hormone to be concentrated in the target tissue. The receptor-hormone complex then acts as a regulatory protein capable of inducing gene expression (Gorski and Gannon, 1976). This investigation seeks to understand the interaction of hormone receptor complex with chromatin. This information will then be applicable to the understanding and treatment of cancer.

In order to study the mechanism of steroid hormone action, a cell-free system should mimic the interaction of steroid receptor with the genes inside the cell. Briefly, the system consists of a receptor-chromatin complex which is digested with micrococcal nuclease. This enzyme was used because it caused cleavage of active genes faster than others (McGhee and Felsenfeld, 1980). Hence, mild digestion of receptor-chromatin com-

## GLOSSARY OF ABBREVIATIONS

For readers who may not be familiar with the medical abbreviations used in the accompanying paper, Dr. Benjamin S. Leung prepared the following glossary:

DNA - Deoxyribonucleic Acid

DNAase - Deoxyribonuclease

EDTA - ethylenediamine tetracetic acid

RNAase - Ribonuclease

S - Svedberg unit

Tris - HCl - Trishydroxylaminomethane-hydrochloride

The frequent number-letter combinations, such as S7, S4, etc., refer to Svedberg units and are used in this paper as identifications coded for research.

plex releases receptors, DNA and other proteins associated with active chromatin. The essential steps in the preparation of cell-free system are described in the following paragraphs.

**Buffers.** TEGD: 10 mM Tris HCl (pH 7.4), 1 mM EDTA, 10 percent glycerol and 1 mM dithiothreitol (DTT). TKM: 50 mM Tris HCl (pH 7.4), 25 mM KCl, and 5 mM MgCl<sub>2</sub>. Micrococcal nuclease buffer: 10 mM Tris HCl (pH 8), 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 1 mM DTT, 25 mM KCl and 0.15 mM spermidine. DNAase I buffer: 10 mM Tris HCl (pH 8), 10 mM NaCl, 3 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, and 1 mM DTT. TE buffer: 10 mM Tris HCl (pH 7.4) and 1 mM EDTA.

**Preparation of chromatin.** Uterine tissue was collected from ovariectomized, estradiol-primed rabbits. Chromatin was extracted at 4°C by the following procedure: The tissue was homogenized with a Polytron (Brinkmann Instruments) in 10 volumes of TKM buffer containing 0.5 M sucrose, filtered through cheesecloth and centrifuged at 10,000 x g for 10 minutes. The nuclear pellet was homogenized with a teflon-glass Potter-Elvehjem homogenizer in TKM buffer containing 2.1 M sucrose, diluted to 1.7 M sucrose, filtered through organza cloth and sedimented at 25,000 x g for 10 minutes. The

\*THRESIA THOMAS, a research associate in the Department of Obstetrics and Gynecology in the University of Minnesota Medical School, presented a summary of this study at the 1982 annual meeting of the Minnesota Academy of Science.

\*\*BENJAMIN S. LEUNG is Associate Professor of Obstetrics and Gynecology in the University of Minnesota Medical School.

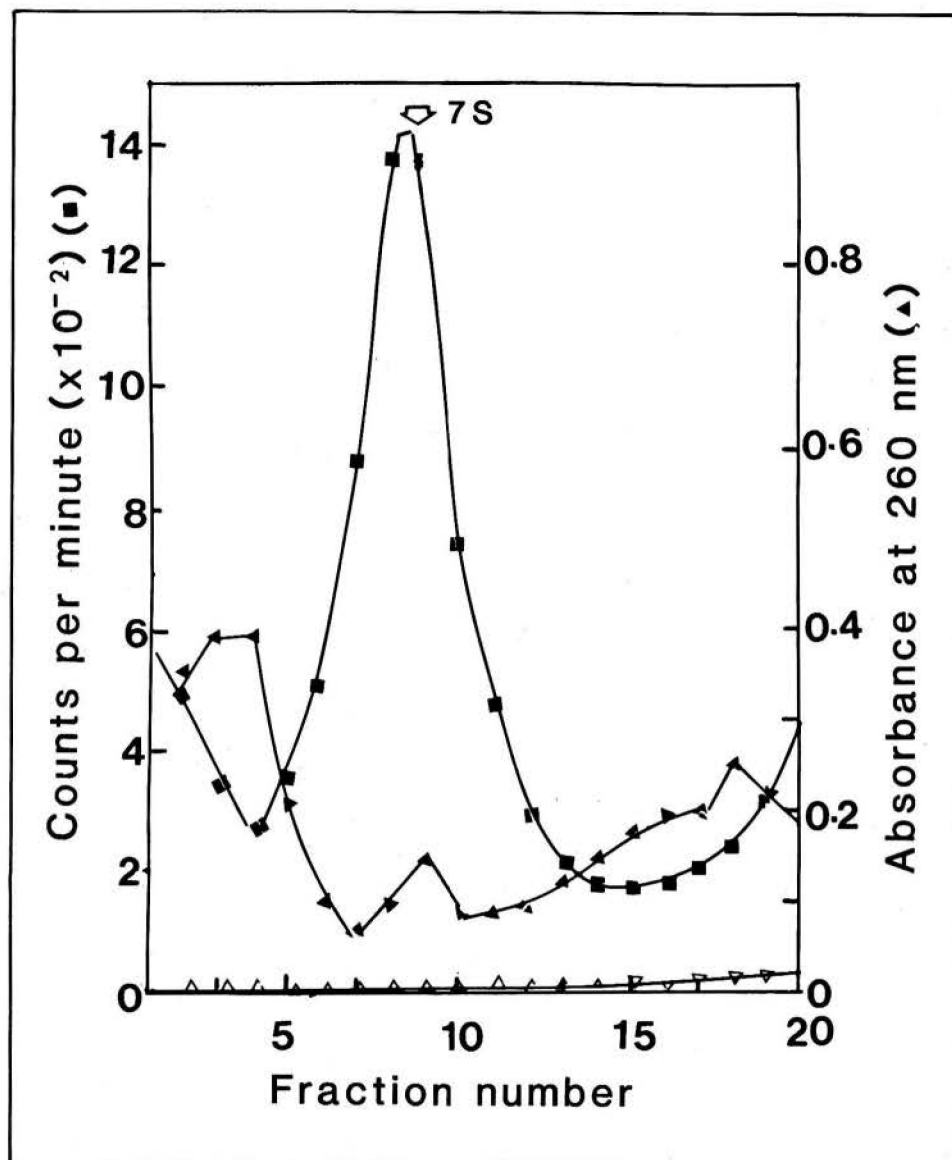


FIGURE 1. - Sucrose density gradient profile of receptors (7 S) released by micrococcal nuclease from receptor-chromatin complex. Symbols used are absorbance (▲), radioactivity (■) and nonspecific binding (▼).

pellet was then washed with TKM buffer containing 0.5M sucrose and 0.2 percent Triton X-100. Chromatin was isolated from the nuclear pellet by a series of homogenizations and sedimentations: (a) 80 mM NaCl and 10 mM EDTA (pH 6.3) three times, (b) 150 mM NaCl and 10 mM sodium citrate (pH 7.0) twice and (c) 1.5 mM NaCl and 0.15 mM sodium citrate (pH 7.0) twice. The chromatin suspension in low salt buffer was stored at  $-70^{\circ}\text{C}$  as 1 ml aliquots containing approximately 1 mg of DNA.

**Preparation of estradiol receptor.** Rabbit uterine tissue was homogenized in TEGD buffer and centrifuged at  $110,000 \times g$  for 1 hr. Estradiol receptors were precipitated by 40 percent saturation of the cytosol with ammonium sulphate. The pellet obtained by centrifugation at  $10,000 \times g$  for 15 minutes was dissolved in TEGD buffer, dialysed for 1 hr (against TEGD buffer) and centrifuged at  $110,000 \times g$  for 30 minutes.

**Preparation of receptor-chromatin complex and nuclease digestion.** The receptor preparation was labeled with 8 nM  $^{125}\text{I}$

beta-(2, 4, 6, 7- $^3\text{H}$ ) estradiol (New England Nuclear, (115 Ci/m-mole)) by incubation at  $0^{\circ}\text{C}$  for 90 minutes. Controls with 100 times excess of diethylstilbestrol (DES) were used to determine nonspecific binding and subsequent release of receptors. Chromatin was homogenized in 10 mM Tris HCl (pH 7.5) to give about 1 mg DNA/ml. Aliquots (one ml) of chromatin were incubated with labeled receptor preparation ( $2-3 \times 10^5$  cpm) for 90 minutes at  $25^{\circ}\text{C}$ . The mixtures were then centrifuged at  $2,000 \times g$  for 10 minutes. The pellets were washed twice with 10 mM Tris HCl (pH 7.5) and resuspended either in micrococcal nuclease buffer or DNAase I buffer. This suspension was treated with 10 microliters of micrococcal nuclease (1 unit/ $A_{260}$ ,  $25^{\circ}\text{C}$ ) (from P.L. Biochemical). The reactions were stopped by the addition of EDTA (10 mM). The mixtures were centrifuged ( $2,000 \times g$ , 20 minutes) and the supernatants were used for sucrose density gradient analysis.

**Sucrose density gradient centrifugation.** Linear sucrose density gradients (10-30 percent) were prepared using Buchler

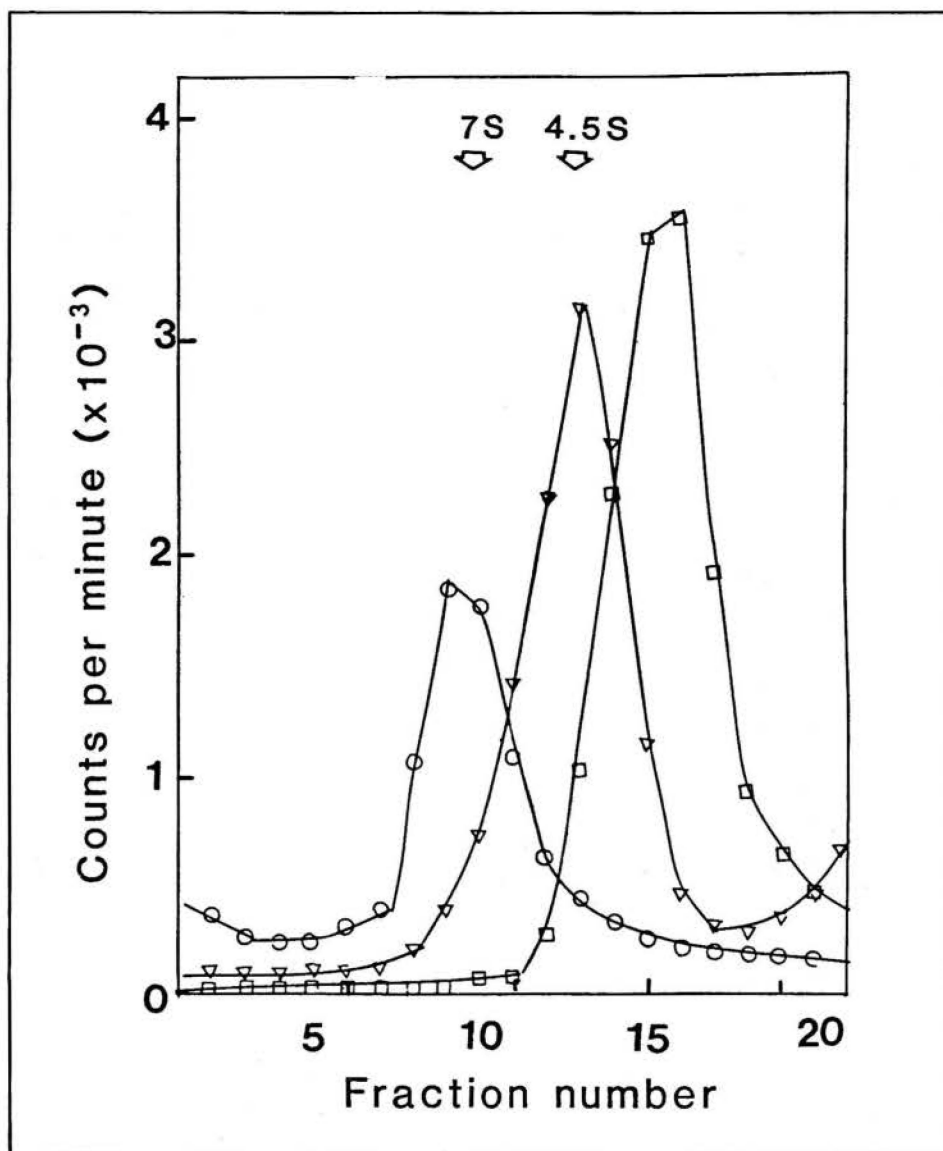


FIGURE 2. - Density gradient profile showing the conversion of 7S receptor (○) to 4.5S in the presence of 0.5 M KCl (Δ) and to 2.8S by digestion with DNAase I (□).

automatic gradient former. TE buffer containing 1 mM DTT and 10 mM NaCl was used. Samples (0.2 ml) were layered on 3.5 ml gradients and centrifuged at 257,000 x g for 15 hours, using a Beckman SW 60 Ti rotor. Internal markers were (<sup>14</sup>C) bovine serum albumin (BSA) and (<sup>14</sup>C) gamma globulin (from New England Nuclear). Mononucleosome, obtained by micrococcal nuclease digestion of chromatin, was sedimented in parallel gradients. Fractions were collected using Buchler fraction collector, and radioactivity of the fractions was determined using Packard Scintillation counter. Mononucleosome was detected by measuring the absorbance at 260 nm of diluted fractions.

#### Properties of Estradiol Receptor

Density gradient analysis is a powerful method that has been successfully used in steroid receptor research. Cytoplasmic receptor sediments at 8S in low salt gradients and 4S in gradients containing 0.5 M KCl. However, receptor extracted from nuclei sediments at 5S both in high and low salt gradients.

The receptor obtained by micrococcal nuclease digestion of the receptor-chromatin complex sedimented at 7S (Figure 1). The fractions collected after gradient centrifugation were monitored for absorbance at 260 nm and a peak was observed in the 7S region. Thus it appeared that DNA or RNA may be associated with the 7S receptor. When the 7S receptor was treated with DNAase I it was shifted to 2.8S (Figure 2). However, RNAase digestion did not affect the sedimentation constant of the receptor. Moreover, another independent method, streptomycin sulphate precipitation, also showed that DNA is indeed bound to the receptor.

DNAase I is another enzyme that attacks active chromatin with high specificity. Digestion of the receptor-chromatin complex with DNAase I produced different forms of receptor: a broad peak (3-7S) at one minute of digestion at 25°C, 5.5S and 3.5S forms at two minutes and 2.8S form at 5 minutes

(not shown). The stepwise reduction in receptor size with DNA hydrolysis, confirms the attachment of DNA to the receptor.

Sedimentation of 7S receptor in high salt gradient caused a shift to 4.5S (Figure 2). Since salt disrupts the non-covalent bonding, it is likely that the binding of 4.5S receptor to DNA is due to ionic interactions. Moreover, since salt dissociation did not produce 2.8S form, it appears that 4.5S to 2.8S conversion is due to a protolytic activity. However, incubation of 8-9S receptor (starting material to form receptor-chromatin complex) with DNAase I did not produce 2.8S form. These results suggest that 4.5S form produced by DNA hydrolysis may be highly susceptible to any protease, either endogeneous or exogeneous, and thus more likely to be converted to 2.8S form.

Estradiol and other steroid receptors are known to bind to chromatin, DNA and other polyanions (Thrall *et al*, 1978). Since chromatin is constituted of DNA, RNA and numerous histone and non-histone proteins, it has been difficult to identify biologically significant binding sites. The results presented in this paper show that the binding of receptor to DNA has high specificity so that the receptor-DNA interactions occur even in the presence of multiple binding sites that exist in chromatin. Biological significance of this binding is underlined by the sensitivity of active genes to micrococcal nuclease and the release of receptor by the enzyme (Thomas and Leung, 1982). Since the method provides an effective way of separating inactive DNA sequences from the receptor binding sequence, it has immense potential in the study of transcriptional control.

## ACKNOWLEDGEMENT

This study was supported by grant from the National Cancer Institute.

## REFERENCES

- GORSKI, J. and GANNON, F. 1976. Current models of steroid hormone action: A critique. *Ann. Rev. Physiol* 38.
- MATHIS, D., OUDET, P. and CHAMBON, P. 1980. Structure of transcribing chromatin. *Progress in Nucleic Acid Research and Molecular Biology* 24.
- McGHEE, J.D. and FELSENFELD, G. 1980. Nucleosome structure. *Ann. Rev. Biochem.* 49.
- O'MALLÉY, B.W., TOWLE, H.C. and SCHWARTZ, R.J. 1977. Regulation of gene expression in eucaryotes. *Ann. Rev. Genet.* 11.
- THOMAS, T. and LEUNG, B.S. 1982. Association of estradiol receptor with DNA of transcriptionally active chromatin. *Nucleic Acids Research* (submitted).
- THRALL, C.L., WEBSTER, R.A. and SPELSBERG, T.C. 1978. Steroid receptor interaction and chromatin. In Busch, H., Editor. *Cell Nucleus, Chromatin Vol., VI, Part C.* Academic Press, New York.

---

## Forthcoming Activities Scheduled by the Academy

EVENT	1983 DATES	PLACE & LEADER
Fall Meeting of the Academy	October 1, 2, 3	Long Lake Conservation Center Palisade, MN. Dr. Wayne Wolsey, Macalester College, Program Chairman
15th Annual Regional Junior Symposium: Science, Engineering, Humanities	November 11 - 13	Quadna Mountain Resort Hill City, MN.
51st Annual Meeting Minnesota Academy of Science	April 29, 30	University of Minnesota, Duluth Dr. Richard Sielaff, Local Chairman

---